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Nile red staining protocol to quantify PHB in Saccharomyces cerevisiae cultivations

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Anna Ylinen[1], Tuula Tenkanen[1]

[1]VTT Technical Research Centre of Finland Ltd

Tuula Tenkanen

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We use this protocol and it's working

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Abstract

Nile red staining can be used to screen PHB producing microbes as it binds on the surface of PHA granules. This protocol has been modified from Spiekermann *et al.* (1999) (DOI 10.1007/s002030050681) and Zuriani *et al.* (2013) (DOI 10.1007/s12257-012-0607-z). We recommend three or four technical replicates for each sample. As a negative control you will need a strain that does not produce PHB or other PHAs as Nile red do not only bind to the surface of PHA granules, but also to other intracellular structures such as lipid droplets.

Before start

Prepare Nile red solution with a concentration of 30 mg/ml in DMSO.

1 Dilute all cultivation samples to same OD with water for example in a 1.5 ml Eppendorf tube. OD
2-5 is preferred but range 0.5-10 works as well. You will need 100 µl of diluted sample/ measurement. Therefore, 400 µl + a little extra is needed for 4 replicate measurements.

2 Pipet 100 µl of each cell sample (all samples now at same OD) to a black 96 well plate.

3 Pipet 20 µl of the Nile red solution (30 mg/ml in DMSO) to each well and mix by pipetting. Incubate for 10 minutes in RT and measure the fluorescence with a fluorescence plate reader.

Protocol references

Spiekermann, P., Rehm, B.H.A., Kalscheuer, R., Baumeister, D., Steinbüchel, A., 1999. A sensitive, viable-colony staining method using Nile red for direct screening of bacteria that accumulate polyhydroxyalkanoic acids and other lipid storage compounds. Archives of Microbiology 171, 73–80. https://doi.org/10.1007/s002030050681

Zuriani, R., Vigneswari, S., Azizan, M.N.M., Majid, M.I.A., Amirul, A.A., 2013. A high throughput Nile red fluorescence method for rapid quantification of intracellular bacterial polyhydroxyalkanoates. Biotechnology and Bioprocess Engineering 18, 472–478. https://doi.org/10.1007/s12257-012-0607-z